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European Journal of Pharmacology 316 (1996) 227–286

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Antioedematogenic and antinociceptive actions of NPC 18521, a novel bradykinin B_2 receptor antagonist

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Received 21 March 1996; revised 12 June 1996; accepted 16 August 1996

Abstract

The novel pseudopeptide bradykinin B_2 receptor antagonist containing the 1,3,8-triazaspiro[4.5]decan-4-one ring system, NPC 18521 (D-Arg-Arg-[1,3-phenyl,8-triazaspiro[4.5]-decane-4-one-3-acetyl]-Ser-D-tetrahydroisoquinolinyl-octahydroindolinyl-Arg) (10 and 30 nmol/kg, i.p.), given 30 min prior, produced significant and long-lasting inhibition of rat paw oedema induced by bradykinin (3 nmol/paw) and carrageenan (300 μg/paw), without affecting the oedema induced by the selective bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, in rats pretreated with *Escherichia coli* endotoxin. In contrast, when injected locally into the rat or mouse hindpaw, NPC 18521 (1–100 nmol) elicited dose-related oedema formation. This effect was almost completely blocked by cyproheptadine (20 mg/kg, i.p.) or by compound 48/80 (12 μg/paw), but was unaffected by Hoe 140 (D-Arg-[Hyp^5,Thi^5,Tic^7,Oic^8]bradykinin). NPC 18521 (0.3–10 nmol/kg, i.p.) produced significant inhibition of acetic acid, acetylcholine and kaolin- but not zymosan-induced abdominal constrictions in mice. The calculated mean ID_{50} values for these effects were 0.84, 0.46 and 0.55 nmol/kg, respectively. The antinociceptive action of NPC 18521 (3 nmol/kg, i.p.) had a rapid onset (15 min) and lasted for up to 120 min. Given topically (0.01–0.3 nmol), NPC 18521 produced significant attenuation of both the early and the late phase of the formalin-induced licking, as well as formalin-induced oedema formation. In addition, NPC 18521 given both systemically or topically, produced significant inhibition of the neurogenic nociception caused by topical injection of capsaicin. Given topically in the rat paw, NPC 18521 (10 nmol) caused marked hyperalgesia, an effect which was completely prevented by cyproheptadine (20 mg/kg, i.p.), but was unaffected by Hoe 140 (3 nmol/kg, i.p.). Given intraperitoneally, 30 min prior, NPC 18521 (3–30 nmol/kg) like Hoe 140 (1–10 nmol/kg) prevented, in a dose-dependent manner, bradykinin (3 nmol/paw)-induced hyperalgesia with mean ID_{50} values of 13.16 and 1.36 nmol/kg, respectively. Thus, the novel pseudopeptide bradykinin B_2 receptor antagonist, NPC 18521, has an effect with rapid onset, and produces potent and relatively long-lasting antioedematogenic and antinociceptive properties. However, in contrast to Hoe 140, given locally into the hindpaw, NPC 18521 elicited marked oedema formation and hyperalgesia, an effect which seems to be secondary to mast cell degranulation and histamine and/or serotonin release. Finally, the anti-bradykinin actions of NPC 18521 are quite selective towards the bradykinin B_2 receptor-mediated responses.

Keywords: Bradykinin; Bradykinin B_1 receptor; Bradykinin B_2 receptor; NPC 18521; Antinociception; Formalin; Capsaicin; Licking; Writhing test; Paw oedema; (Mouse); (Rat)

1. Introduction

The nonapeptide, bradykinin (Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg), and the decapeptide, kallidin (lysyl-bradykinin), are endogenous peptides generated from plasma and tissues, from high and low molecular kininogens respectively, by the action of serine protease kallikreins. Kinins are vasoactive peptides which have an important role as inflammatory mediators and are normally released following tissue trauma or infection. Kinins also excite Aδ and C fibres in sensory neurones, producing nociception and hyperalgesia. In addition, kinins can release pro-inflammatory mediators such as neuropeptides

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PII S0014-2999(96)00661-9

and can also activate phospholipase A_2, resulting in the release of metabolites derived from arachidonic acid pathways which also contribute largely to their inflammatory and nociceptive properties (see, for review, Hall, 1992; Farmer and Burch, 1992; Bhoola et al., 1992; Geppetti, 1993; Dray and Perkins, 1993; Marceau, 1995).

The actions of kinins are mediated through stimulation of two subtypes of membrane receptors, B_1 and B_2. The kinin B_1 receptors exhibit higher affinity for the kinin active carboxypeptidase metabolites, des-Arg^9-bradykinin and des-Arg^{10}-kallidin. The kinin B_1 receptors are rarely expressed in non-traumatized tissues, but can be induced following tissue trauma or infection, and are specifically antagonized by the selective bradykinin B_1 receptor antagonists, des-Arg^9[Leu^8]bradykinin and des-Arg^{10}[Leu^8]kallidin. On the other hand, bradykinin and kallidin have great affinity for the kinin B_2 receptors, which are constitutively and widely distributed throughout central and peripheral tissues. Thus, the majority of physiological kinin actions seem to be mediated by stimulation of constitutive kinin B_2 receptors, being competitively antagonized by several selective bradykinin B_2 receptor antagonists (Hall, 1992; Farmer and Burch, 1992). So far, the kinin B_1 and B_2 receptors have been cloned; they are members of the superfamily of guanine nucleotide binding proteins (G proteins)-coupled receptors (McEachern et al., 1991; Hess et al., 1992; Hess et al., 1994; Eggerickx et al., 1992; Powell et al., 1993; Menke et al., 1994).

In recent years, considerable progress has been made with the development of selective and specific bradykinin B_2 receptor antagonists (see, for review, Hall, 1992; Farmer and Burch, 1992; Bhoola et al., 1992; Kyle, 1995). By using both such peptide antagonists and selective agonists, it has been possible to characterize the mechanisms and receptor subtypes for kinin in most tissues. Furthermore, the development of the second generation of bradykinin B_2 receptor antagonists, such as Hoe 140 (D-Arg-[Hyp^5,Thi^5,Tic^7,Oic^8]bradykinin), NPC 17731 (D-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-D-Hype[trans propyl]-Oic-Arg) and NPC 17761 (D-Arg-Arg-Pro-Hyp-Gly-Phe-Ser-D-Hyp[*trans*-thiophenyl]-Oic-Arg) (Hock et al., 1991; Wirth et al., 1991; Kyle and Burch, 1992; Kyle, 1994), has provided useful tools for elucidating the physiological and pathological role of kinins in most systems.

The aim of the present study was to evaluate the topical and systemic antioedematogenic and antinociceptive properties of the novel pseudopeptide bradykinin B_2 receptor antagonist, NPC 18521 (D-Arg-Arg-[1,3-phenyl,8-triazaspiro[4.5]-decane-4-one-3-acetyl]-Ser-D-tetrahydroisoquinolinyl-octahydroindolinyl-Arg) (Fig. 1) (Mavunkel et al., 1996).

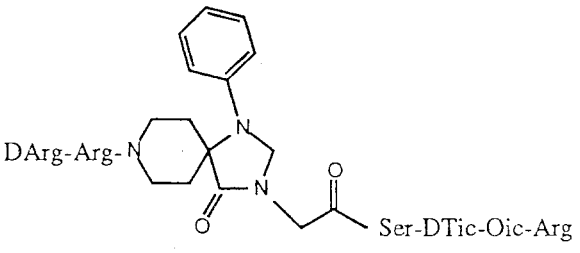

Fig. 1. Molecular structure of NPC 18521.

2. Materials and methods

2.1. Animals

Non-fasted Swiss male Wistar rats (120–150 g) or mice (18–30 g) from our department, housed at 22°C with a 12 h:12 h light-dark cycle, were used. Food and water were freely available. The experiments reported were carried out in accordance with current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

2.2. Measurement of rat and mouse paw oedema

In experiments with bradykinin, the animals were pretreated with the angiotensin-converting enzyme inhibitor, captopril (5 mg/kg, s.c.), 1 h prior to experiments, to prevent the degradation of bradykinin (Corrêa and Calixto, 1993). The animals were lightly anaesthetised with ether and received 100 μl (rats) or 50 μl (mice) intraplantar injections in one hindpaw of phosphate-buffered saline (phosphate-buffer solution; composition mmol/l: NaCl 137; KCl 2.7 and phosphate buffer 10) containing bradykinin (3 nmol), des-Arg^9-bradykinin (100 nmol), carrageenan (300 μg/paw) alone or in combination with NPC 18521 (3 and 10 nmol) or Hoe 140 (1–10 nmol). Since kinin B_1 receptors are not constitutively present, oedema elicited by the bradykinin B_1 receptor agonist, des-Arg^9-bradykinin, was provoked in animals that received *Escherichia coli* endotoxin (10 μg/kg, i.v.) 24 h prior to testing, as described previously (Campos et al., 1995, 1996). In a separate group of experiments, NPC 18521 (10 and 30 nmol/kg) or Hoe 140 (3 and 10 nmol/kg) was administered systemically i.p., 30 min before the intraplantar injection of the phlogistic agents. The contralateral paws received a similar volume of phosphate-buffer solution and were used as control. Oedema was measured with a plethysmometer (Ugo Basile) at several time points after injection of phlogistic agent. Oedema formation was expressed as ml (rats) or μl (mice).

In order to investigate the role of histamine and/or serotonin in the residual agonistic activity of NPC 18521, other groups of animals were treated with cyproheptadine (20 mg/kg, i.p., 30 min prior) (Steranka and Burch, 1991), or with compound 48/80 (12 μg/paw), 24 h prior to experiments (Martins et al., 1990).

2.3. Abdominal constriction response caused by intraperitoneal injection of acetic acid, acetylcholine, zymosan or kaolin in mice

The abdominal constrictions resulting from intraperitoneal injection of acetic acid (0.6%), acetylcholine (5 mg/kg), kaolin suspension (50 mg/kg) or zymosan suspension (40 mg/kg), consisting of a contraction of the abdominal muscle together with a stretching of hind limbs, were induced according to procedures described previously (Collier et al., 1968; Corrêa et al., 1996). Mice were pretreated with NPC 18521 (0.3–10 nmol/kg, i.p.) 15–150 min before the irritant injections. Control animals received a similar volume of 0.9% NaCl solution (10 ml/kg, i.p.). All experiments were carried out at 20–22°C. After challenge, pairs of mice were placed in separate boxes, and the abdominal constrictions were counted cumulatively over a period of 20 min for acetic acid and kaolin, 15 min for zymosan and 10 min for acetylcholine. Antinociceptive activity was expressed as the reduction of the number of abdominal constrictions, i.e., the difference between control animals (saline-pretreated mice) and animals pretreated with NPC 18521 or with Hoe 140, which were used as a positive control.

2.4. Formalin-induced licking in mice

The procedure was similiar to that described previously (Corrêa and Calixto, 1993). Animals from the same strain were lightly anaesthetised with ether, except when used to analyse the early phase of formalin-induced licking, and 20 μl of 2.5% formalin (0.92% formaldehyde) made up in phosphate-buffer solution was injected under the surface of the right hindpaw. Two mice (control and treated) were observed simultaneously from 0 to 30 min following formalin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The early phase of the nociceptive response normally peaked 5 min after formalin injection and the late phase 15–30 min after formalin injection, representing the tonic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). Animals received NPC 18521 (0.3–10 nmol/kg, i.p.), 30 min beforehand or locally (0.01–0.3 nmol/paw) together with formalin injection, respectively. Control animals received only the vehicle used to dilute NPC 18521 (NaCl solution, 10 ml/kg, i.p., or 20 μl/paw of phosphate-buffer solution). Following intraplantar injection of formalin, the animals were immediately placed into a glass cylinder 20 cm in diameter, and the time spent licking the injected paw was determined.

To investigate whether the antinociceptive activity of NPC 18521 on formalin-induced licking was associated with antioedematogenic activity, we measured the paw oedema by comparing the difference in weight of the formalin-treated paw and of the control paw (phosphate-buffer solution-treated paw). For this purpose, animals were killed 30 min after formalin injection by cervical dislocation, and the paw was cut at the knee joint and weighed on an analytical balance. In order to analyse the mechanism underlying the residual agonistic activity of NPC 18521 when it was injected locally into the hindpaw, some groups of animals were treated with compound 48/80 (12 μg/paw) 24 h prior to NPC 18521 injection.

2.5. Capsaicin-induced licking in mice

The animals were placed individually in transparent glass cylinders of 20 cm in diameter, which served as observation chambers. Following the adaptation period, 20 μl of capsaicin (1.6 μg/paw made in phosphate-buffer solution) was injected under the skin of the dorsal surface of the right hindpaw using a microsyringe with a 26 gauge needle. The contralateral paw received a similar volume of phosphate-buffer solution. The procedure used was similar to that described previously (Sakurada et al., 1992; Corrêa et al., 1996). Mice were observed individually for 5 min following capsaicin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The animals were treated with NPC 18521 (0.3–10 nmol/kg, i.p.) 30 min beforehand, or locally (0.01–0.3 nmol) together with capsaicin injection, respectively. Control animals received a similar volume of 0.9% NaCl (10 ml/kg, i.p. or 20 μl/paw of phosphate-buffer solution). As described for the formalin model, some animals were pretreated with compound 48/80 (12 μg/paw) 24 h before the test.

2.6. Effect of NPC 18521 and Hoe 140 on bradykinin-induced hyperalgesia in the rat paw

The animals were pretreated with the angiotensin-converting enzyme inhibitor, captopril (5 mg/kg, s.c.), 1 h prior to experiments, to prevent the degradation of bradykinin (Corrêa and Calixto, 1993). The animals received an intraplantar injection of 0.1 ml of bradykinin (3 nmol), NPC 18521 (10 and 30 nmol), Hoe 140 (1–10 nmol) or only phosphate-buffer solution into the right hindpaw and the hyperalgesic effect was assessed 30 min later. In another group of experiments, the animals received NPC 18521 (3–30 nmol/kg) or Hoe 140 (1–10 nmol/kg) intraperitoneally 30 min before bradykinin challenge. Similarly, Hoe 140 (3 nmol/kg, i.p.) or cyproheptadine (20 mg/kg, i.p.) was administered 30 min prior to topical injection of NPC 18521 (10 nmol/paw), in order to analyse the mechanism underlying the residual agonistic activity of NPC 18521.

The nociceptive threshold (to squeak response or paw withdrawal) was assessed by applying increasing pressure to the dorsal site of a control or bradykinin (3 nmol)-injected rat hindpaw, using a modified Basile analgesy meter (Ugo Basile, Milan, Italy) according to the method of

Randall and Selitto (1957). The weight on the analgesy meter ranged from 0 to 750 g, and the threshold was expressed as load (grams) tolerated.

2.7. Drugs

The drugs used were: NPC 18521 (D-Arg-Arg-[1,3-phenyl,8-triazaspiro[4.5]-decane-4-one-3-acetyl]-Ser-D-tetrahydroisoquinolinyl-octahydroindolinyl-Arg) (Scios Nova, Sunnyvale, USA), formalin, acetic acid (Merck, Darmstadt, Germany), zymosan, acetylcholine, carrageenan lambda grade IV, cyproheptadine, compound 48/80, bacterial lipopolysaccharide (*Escherichia coli* serotype 0111, L = 2630) (all from Sigma, St. Louis, MO, USA), kaolin (Wako Pure Chemical Industries, kindly supplied by Dr T. Fujiyoshi, Kitasato University, Minato-ku, Tokyo, Japan), des-Arg^9-bradykinin (Peninsula, Belmont, CA, USA), capsaicin (Calbichem, San Diego, CA, USA) and Hoe 140 [D-Arg-(Hyp^3,Thy^5,D-Tic^7,Oic^8]bradykinin, which was kindly supplied by Hoechst (Frankfurt am Main, Germany). All other reagents used were of a high grade of purity. The stock solutions for NPC 18521 or Hoe 140 (1 mM), kept in siliconized plastic tubes, were held in the freezer at −18°C. The other drugs were prepared just before use in 0.9% (w/v) NaCl solution, except capsaicin, which was dissolved in ethanol. The final concentration of ethanol did not exceed 5% and did not itself cause any effect.

2.8. Statistical analysis

The results are presented as means ± S.E.M., except the ID_{50} or ED_{50} values (i.e., the doses of antagonists necessary to reduce the response by 50% relative to the control value or to the dose of agonist needed to cause a half-maximal response, respectively), which are reported as geometric means accompanied by their respective 95% confidence limits. The statistical significance of differences between groups was obtained by means of one-way analysis of variances followed by Dunnett's multiple comparison test (for analgesia data) and by means of two-way analysis of variances followed by the ad hoc Bonferroni test (for oedema data), when appropriate. P values less than 0.05 were considered as indicative of significance. The ID_{50} or ED_{50} values were estimated from individual experiments by using the least squares method via graphical interpolation.

3. Results

3.1. Effect of NPC 18521 on bradykinin- and carrageenan-induced rat and mouse paw oedema

The treatment of rats or mice with NPC 18521 (10 and 30 nmol/kg), given intraperitoneally 30 min before, caused a significant inhibition of bradykinin (3 nmol)-induced oedema formation (Fig. 2A,C), with maximal inhibitions (mean ± S.E.M.) of 53 ± 4 and 47 ± 4%, respectively.

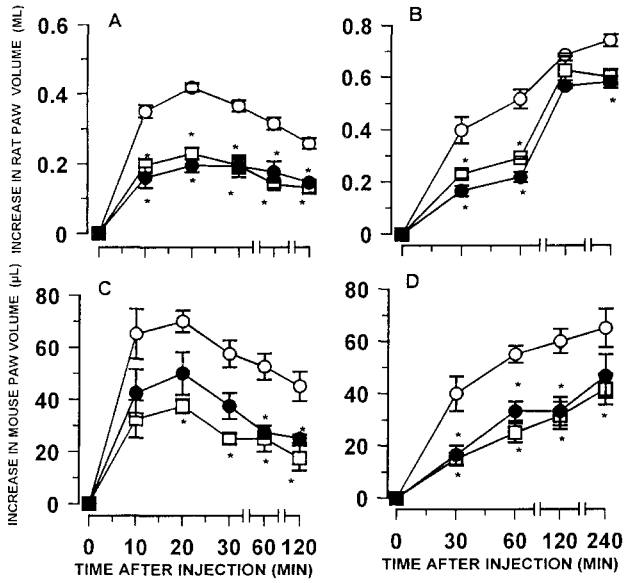

Fig. 2. Effect of systemic treatment with NPC 18521 (● 10 and □ 30 nmol/kg, i.p., 30 min) on bradykinin (3 nmol/paw)-induced oedema in rat (panel A) or mouse (panel C) and on carrageenan (300 µg/paw)-induced rat (panel B) or mouse (panel D) paw oedema. The control responses are shown in all panels (○). Each point represents the mean ± S.E.M. for 6 animals. In some cases the error bars of the mean are hidden within the symbols. Significantly different from control values: * $P < 0.05$.

Similarly, the carrageenan (300 µg)-induced oedema formation (Fig. 2B,D) was significantly inhibited by NPC 18521 (10 and 30 nmol/kg, i.p.), with maximal inhibition at 30 min (mean ± S.E.M.) of 62 ± 4 and 69 ± 8%, respectively, for rats and mice. The paw oedema inhibition caused by NPC 18521 in both species was relatively long-lasting (2–4 h) ($P < 0.05$) (Fig. 2A–D). In animals pretreated with cyproheptadine (20 mg/kg, i.p.), 30 min prior, NPC 18521 (3 and 10 nmol/paw) also caused significant though non-dose-related inhibition of bradykinin-induced rat paw oedema (Fig. 3A). The maximal oedema inhibition (mean ± S.E.M.) was 49 ± 5%.

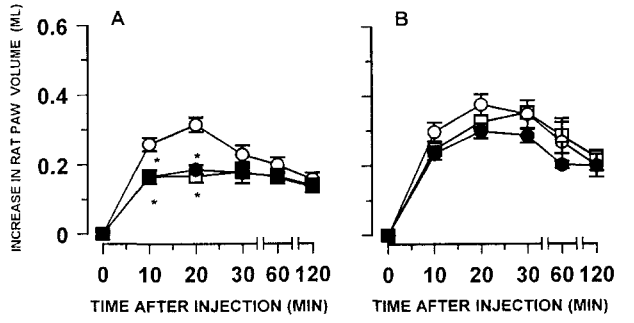

Fig. 3. (A) Effect of intraplantar injection of NPC 18521 (● 3 and □ 10 nmol/paw) on bradykinin (○ 3 nmol/paw)-induced rat paw oedema in the presence of cyproheptadine (20 mg/kg, i.p., 30 min). (B) Effect of systemic treatment with NPC 18521 (● 10 and □ 30 nmol/kg, i.p., 30 min) on des-Arg^9-bradykinin (○ 100 nmol/paw)-induced rat paw oedema. Each point represents the mean ± S.E.M. for 6 animals. In some cases the error bars of the mean are hidden within the symbols. Significantly different from control values: * $P < 0.05$.

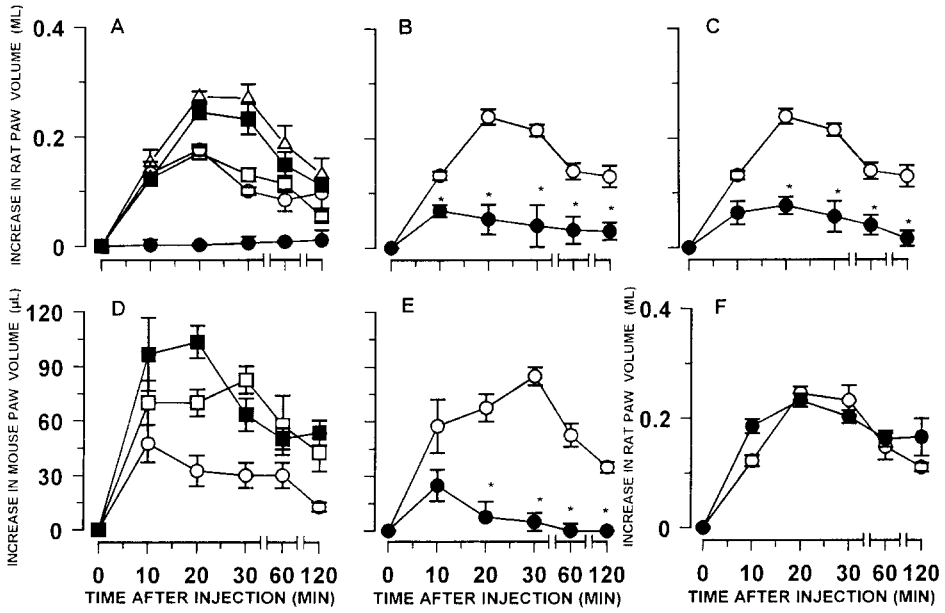

Fig. 4. Time- and dose-response curves showing the increase in rat (panel A) and mouse (panel D) paw oedema caused by intraplantar injection of NPC 18521 (● 1; ○ 3; □ 10; ■ 30 and △ 100 nmol/paw; panel A). Effects of pretreatment with cyproheptadine (● 20 mg/kg, i.p., 30 min, panel B) or with compound 48/80 (● 12 μg/paw, 24 h, panel C) and effect of co-injection of HOE 140 (● 3 nmol/paw, panel F) on NPC 18521-induced rat paw oedema (○ 30 nmol/paw). Effect of pretreatment with cyproheptadine (● 20 mg/kg, i.p., 30 min, panel E) on NPC 18521-induced mouse paw oedema. Each point represents the mean ± S.E.M. for 5 animals. In some cases the error bars of the mean are hidden within the symbols. Significantly different from control values: * $P < 0.05$.

Interestingly, up to 30 nmol/kg, i.p., NPC 18521 did not significantly affect des-Arg^9-bradykinin-mediated paw oedema in rats which had been previously treated with *Escherichia coli* endotoxin (Fig. 3B).

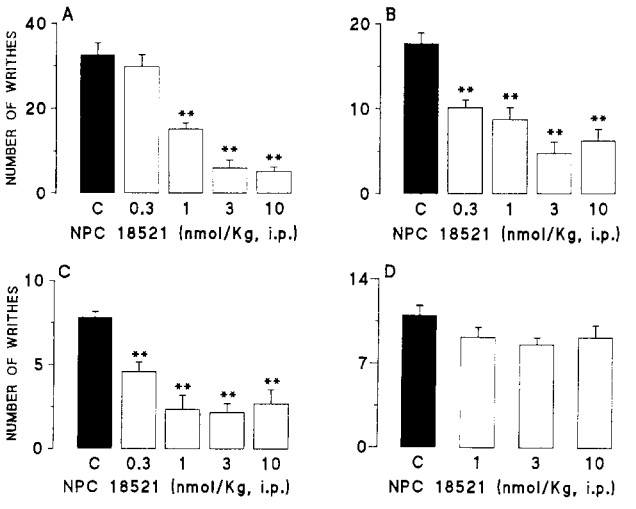

Fig. 5. Effect of systemic treatment of animals with NPC 18521 on the writhing responses induced by intraperitoneal injection of acetic acid (panel A), kaolin (panel B), acetylcholine (panel C) and zymosan (panel D) in mice. The total number of writhes (mean ± S.E.M.) was measured during the first 20 min (acetic acid and kaolin), 15 min (zymosan) or 10 min (acetylcholine) following intraperitoneal injection of the irritants. Each column represents the mean for 8 to 10 animals and the vertical bars indicate the S.E.M. The closed columns indicate the control values (C) (animals injected with irritant without antagonist) and the asterisks denote the significance levels. Significantly different from control values, * $P < 0.05$; ** $P < 0.01$.

When NPC 18521 was injected locally into rat or mouse hindpaws (3–100 nmol), it produced a dose-related oedema formation (Fig. 4A,D). In both species, the residual agonistic activity of NPC 18521 was almost completely prevented by previous treatment of the animals with cyproheptadine (20 mg/kg, i.p., 30 min) (Fig. 4B,E). Similar inhibition was also observed in rats pretreated with compound 48/80 (12 μg/paw) 24 h beforehand (Fig. 4C). However, the rat paw oedema formation induced by NPC 18521 was unaffected by Hoe 140 (3 nmol/paw) (Fig. 4F).

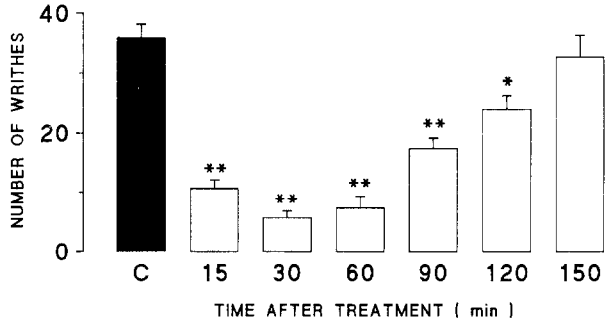

Fig. 6. Time-course for the antinociceptive effect of systemic treatment of animals with NPC 18521 (3 nmol/kg, i.p.) on the writhing responses induced by intraperitoneal injection of acetic acid in mice. The total number of writhes (mean ± S.E.M.) was measured during the first 20 min after injection of acetic acid. Each column represents the mean for 8 to 10 animals and the vertical bars indicate the S.E.M. The closed columns indicate the control values (C) (animals injected with acetic acid without antagonist) and the asterisks denote the significance levels. Significantly different from control values, * $P < 0.05$; ** $P < 0.01$.

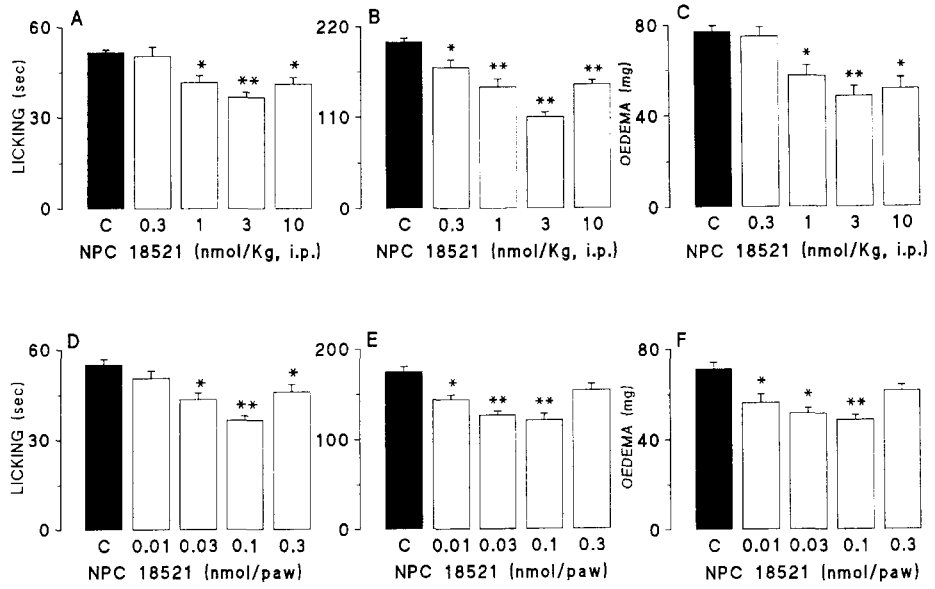

Fig. 7. Effect of systemic (panels A and B) or topical (panels D and E) treatment of animals with NPC 18521 on formalin-induced nociception and formalin-induced paw oedema (panels C and F) in mice. The total time (mean ± S.E.M.) spent licking the hindpaw was measured in the early (0–5 min, panels A and D) and the late phase (15–30 min, panels B and E), after intradermal injection of formalin in the hindpaw. Each column represents the mean for 8 to 10 animals and the vertical bars indicate the S.E.M. The closed columns indicate the control values (C) (animals injected with formalin without the antagonist) and the asterisks denote significance levels. Significantly different from control values, * $P < 0.05$; ** $P < 0.01$.

3.2. Antinociceptive effects of NPC 18521

Results of Fig. 5(A–C) show that the pretreatment of animals with intraperitoneal doses of NPC 18521 (0.3–10 nmol/kg), 30 min beforehand, resulted in a graded and significant inhibition of acetic acid, acetylcholine and kaolin-induced writhing responses in mice. The estimated mean ID_{50} values (and their 95% confidence limits) and the maximal inhibitions (mean ± S.E.M.) for these effects were: 0.84 (0.74–0.96); 0.46 (0.27–0.78) and 0.55 (0.44–0.68) nmol/kg and 80 ± 3; 72 ± 7 and 70 ± 5%, against acetic acid, acetylcholine and kaolin, respectively. However, at the same doses, NPC 18521 did not affect significantly the abdominal constrictions induced by zymosan (Fig. 5D).

Fig. 6 shows the time-course for the antinociceptive action of NPC 18521 (3 nmol/kg) given intraperitoneally, against the acetic-acid-induced writhing response. The antinociception of NPC 18521 appeared within the first 15 min following its intraperitoneal injection, and lasted for at least 120 min.

The NPC 18521 (0.3–10 nmol/kg) given i.p. 30 min prior also prevented significantly, though not in a dose-related manner, the late phase of the formalin-induced licking, and, to a lesser extent, the neurogenic component (early phase) of the formalin-induced nociception (Fig. 7A,B). The maximal inhibitions (mean ± S.E.M.) of the formalin response caused by NPC 18521 were 29 ± 3 and 45 ± 3% respectively, against the early and the late phases. In addition, NPC 18521 at the same doses also produced a significant inhibition of the paw oedema formation associated with the late phase of the formalin-induced nociception, with maximal inhibition (mean ± S.E.M.) of 40 ± 6% at the dose of 3 nmol/kg ($P < 0.05$) (Fig. 7C).

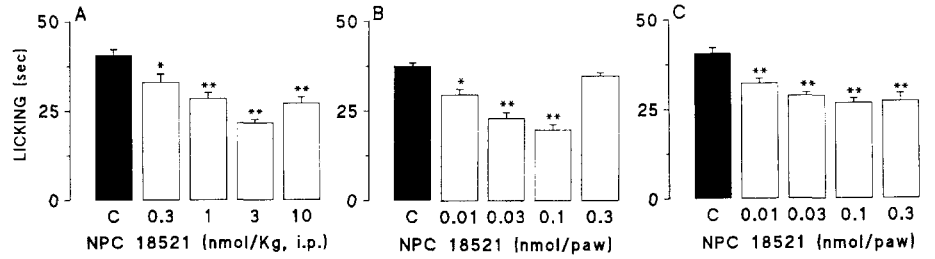

Fig. 8. Effect of intraperitoneal (panel A) or topical (panel B) injection of NPC 18521 on the capsaicin-induced licking in mice. The panel C shows the influence of the pretreatment with compound 48/80 (12 μg/paw, 24 h prior) on the antinociceptive effect of NPC 18521 on capsaicin-induced pain. The total time (mean ± S.E.M.) spent licking the hindpaw (0–5 min) was measured after intradermal injection of capsaicin in the hindpaw. Each column represents the mean for 8 to 10 animals and the vertical bars indicate the S.E.M. The closed columns indicate the control values (C) (animals injected with capsaicin without antagonist) and the asterisks denote the significance levels. Significantly different from control values, * $P < 0.05$; ** $P < 0.01$.

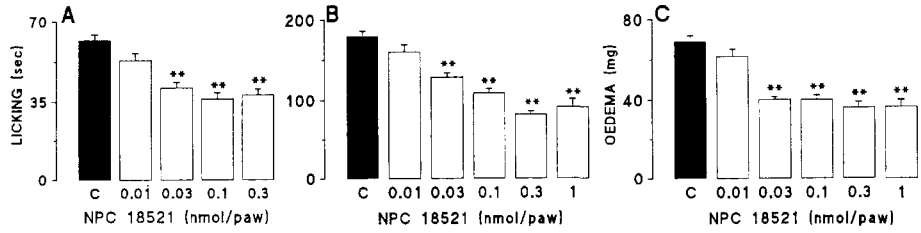

Fig. 9. Influence of the pretreatment of animals with compound 48/80 (12 μg/paw, 24 h prior) on the antinociceptive effect of NPC 18521 on formalin-induced nociception (panels A and B) and formalin-induced paw oedema (panel C) in mice. The total time (mean ± S.E.M.) spent licking the hindpaw was measured in the early (0–5 min, panel A) and the late phase (15–30 min, panel B), after intradermal injection of formalin into the hindpaw. Each column represents the mean for 6 to 8 animals and the vertical bars indicate the S.E.M.. The closed columns indicate the control values (C) (animals injected with formalin without antagonist) and the asterisks denote significance levels. Significantly different from control values, ** $P < 0.01$.

When injected locally in association with formalin, NPC 18521 (0.01–0.3 nmol) caused a significant inhibition of both phases of the formalin-induced licking (Fig. 7D,E). The maximal inhibitions (mean ± S.E.M.) of the responses were 34 ± 3 and 31 ± 4%, respectively. NPC 18521 (0.01 and 0.3 nmol) also antagonized partially, but significantly, the paw oedema associated with the late phase of the formalin-induced nociception, with a maximal inhibition (mean ± S.E.M.) of 32 ± 3% (Fig. 7F). In addition, when given locally (0.01–0.1 nmol/paw) or i.p. (0.3–10 nmol/kg, 30 min prior), NPC 18521 produced a graded attenuation of the nociceptive response caused by intraplantar injection of capsaicin (1.6 μg/paw). The maximal inhibitions (mean ± S.E.M.) of the responses were 47 ± 4 and 47 ± 3%, respectively (Fig. 8A,B). NPC 18521, similar to other bradykinin peptides receptor antagonists, demonstrated a residual agonistic activity in both experimental models of pain. In the case of both capsaicin (Fig. 8C) and formalin (Fig. 9)-induced licking, the residual agonistic activity caused by higher concentrations of NPC 18521 was fully prevented by previous treatment of the animals with compound 48/80 (12 μg/paw), 24 h prior to the experiments.

When injected locally, bradykinin (1–10 nmol) caused a dose-related hyperalgesic effect, with mean ED_{50} (and 95% confidence limits) and maximal effect of 1.85 (0.8–4.26) nmol/paw and 64 ± 4%, respectively ($n = 6$) (results not shown). Similarly, topical injection of NPC 18521 (10 nmol) caused a significant hyperalgesic response (control paw 371 ± 27 of tolerated load g versus 167 ± 12 g in presence of NPC 18521) ($P < 0.01$) ($n = 6$) (not shown). At a higher concentration, NPC 18521 (30 nmol/paw) failed to induce hyperalgesia (results not shown). The hyperalgesic response induced by NPC 18521 (10 nmol) was not affected significantly by intraperitoneal treatment of animals with Hoe 140 (3 nmol/kg) (from 203 ± 22 g in untreated animals to 226 ± 18 g in animals pretreated with Hoe 140) ($P > 0.05$) ($n = 6$). However, cyproheptadine (20 mg/kg, i.p.) completely prevented the hyperalgesic action induced by local injection of NPC 18521 (10 nmol) (response to NPC 18521 alone of 203 ± 22 of tolerated load g versus 398 ± 22 of tolerated load g in animals treated with cyproheptadine) ($P < 0.01$) ($n = 6$). Both NPC 18521 (3–30 nmol/kg) and Hoe 140 (1–10 nmol/kg), given i.p., dose-dependently reversed the hyperalgesic effect caused by intraplantar injection of bradykinin (3 nmol) (Fig. 10A,B). Hoe 140 was about 10-fold more potent than NPC 18521, with mean ED_{50} values (and 95% confidence limits) of 1.36 (0.5–3.69) and 13.16 (11.34–15.27) nmol/kg, respectively.

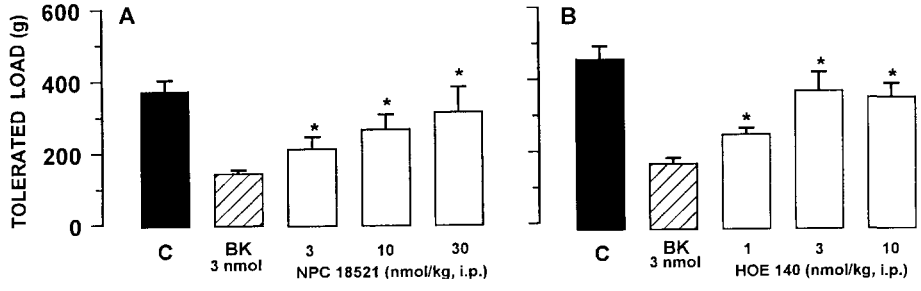

Fig. 10. Direct dose-related anti-hyperalgesic actions of NPC 18521 (3–30 nmol/kg, i.p., panel A, open bars) or HOE 140 (1–10 nmol/kg, i.p., panel B, open bars) on bradykinin (3 nmol/paw)-induced hyperalgesia in the rat paw. The closed bars represent the phosphate buffer solution-injected paws and the hatched bars the bradykinin (3 nmol)-injected paws. Each column represents the mean ± S.E.M for 5 animals. Significantly different from control values: * $P < 0.05$.

4. Discussion

Recently, a new series of pseudopeptide bradykinin B_2 receptor antagonists has been proposed on the basis of the seven transmembrane rodopsin receptors and experimental mutagenesis analysis. Some of these pseudopeptides, including NPC 18688 (D-Arg-Arg-[1,3-cyclohexyl-8-triazaspiro[4.5]-decane-4-one-3-acetyl]-Ser-D-tetrahydroisoquinolinyl-octahydroindolinyl-Arg) and NPC 18521, exhibited higher affinity for the human bradykinin B_2 receptor and were able to block bradykinin-mediated actions in vivo (Kyle, 1994; Chakravarty et al., 1995; Mavunkel et al., 1996).

In an earlier study, we had demonstrated that the new pseudopeptide bradykinin B_2 receptor antagonist NPC 18688, structurally analogous to NPC 18521, given either topically or systemically, produced significant and long-lasting anti-hyperalgesic action when evaluated in several models of nociception in mice (Corrêa et al., 1996). NPC 18688 was devoid of residual agonistic activity, and its actions were quite selective in inhibiting bradykinin B_2 receptor-mediated response. In addition, NPC 18688 and Hoe 140, given either intraperitoneally or topically in association with bradykinin, produced a dose-related antiedematogenic action in rat and mouse hindpaws, although NPC 18688 was about 10-fold less potent than Hoe 140 (Corrêa et al., 1996).

The results of the present study showed that the novel pseudopeptide bradykinin B_2 receptor antagonist, NPC 18521, administered systemically to rats and mice, elicited a significant and dose-related antioedematogenic effect. NPC 18521 was about 2-fold more potent than NPC 18688, although it was 6-fold less potent than Hoe 140 (Corrêa et al., 1996). In marked contrast to the effect reported for Hoe 140, NPC 18688, NPC 17731 and NPC 17761 (Corrêa and Calixto, 1993; Corrêa et al., 1996), when NPC 18521 was injected locally into rat or mouse hindpaw, it elicited a pronounced and dose-related residual agonistic activity. Similar results have been demonstrated previously for the first generation of bradykinin B_2 antagonists (Steranka et al., 1988a,b; Kindgen-Milles and Klement, 1992; Corrêa and Calixto, 1993). The agonistic effect of NPC 18521 seems to be secondary to mast cell degranulation and histamine and/or serotonin release. These observations are substantiated by the results showing that the previous systemic treatment of mice or rats with cyproheptadine, or topically with compound 48/80, completely prevented the residual agonistic effect in response to topical injection of NPC 18521. An additional piece of evidence indicating that this effect does not involve activation of bradykinin B_2 receptors was supplied by the results showing that Hoe 140, at doses at which it exhibits pronounced attenuation of bradykinin-induced paw oedema (Campos and Calixto, 1995; Corrêa et al., 1996; Campos et al., 1995, 1996), had no effect on NPC 18521-mediated oedema formation. There are also relevant results demonstrating that, in both animal species, NPC 18521, as reported for other bradykinin B_2 receptor antagonists (Burch and DeHaas, 1990; Wirth et al., 1991; Costello and Hargreaves, 1989; Damas et al., 1990; Damas and Remacle-Volon, 1992), causes significant and long-lasting inhibition of carrageenan-induced oedema formation. Such results further confirm the notion that kinins have an important role in carrageenan-induced oedema formation (Burch and DeHaas, 1990; Wirth et al., 1991; Costello and Hargreaves, 1989; Damas et al., 1990; Damas and Remacle-Volon, 1992). As demonstrated previously for other bradykinin B_2 receptor antagonists, such as Hoe 140, NPC 17731, NPC 17761 and NPC 18688 (Lembeck et al., 1991; Hock et al., 1991; Campos and Calixto, 1995; Campos et al., 1995, 1996; Corrêa et al., 1996), the antioedematogenic action of NPC 18521 was quite selective for the bradykinin B_2 receptor, evident by the fact that NPC 18521, at doses where it consistently inhibits bradykinin-mediated oedema formation, does not cause any significant inhibition of oedema formation induced by the bradykinin B_1 receptor agonist des-Arg^9-bradykinin in rats previously treated with *Escherichia coli* endotoxin (Campos et al., 1995, 1996).

When assessed in several models of nociception, NPC 18521, given systemically, dose dependently inhibits acetic acid-, kaolin- and acetylcholine-induced abdominal constrictions in mice. On a nmol basis, NPC 18521 is about 15-fold more potent than Hoe 140 and about 90-fold more active than NPC 18688 (Corrêa et al., 1996). Interestingly, NPC 18521, in contrast to what was reported for its analogue, NPC 18688 (Corrêa et al., 1996), did not significantly discriminate between the nociception induced by the three substances. The reason why NPC 18521 was more potent than Hoe 140 to induce antinociception, but not to inhibit paw oedema still remains unclear and was not further investigated in the present study. In addition, the systemic anti-hyperalgesic action of NPC 18521, as reported previously for NPC 18688, occurred rapidly and lasted for up to 120 min. Such results reinforce previous data suggesting that kinins may play an important role in the nociception induced by the intraperitoneal injection of acetic acid, kaolin and acetylcholine in mice (Heapy et al., 1993; Corrêa et al., 1996). As reported previously for Hoe 140 (Heapy et al., 1993), NPC 18521 also failed to prevent zymosan-induced abdominal constrictions, suggesting a lack of involvement of kinins in this model of pain.

As reported previously for bradykinin B_2 receptor antagonists (Haley et al., 1989; Shibata et al., 1986; Chapman and Dickenson, 1992; Corrêa and Calixto, 1993; Corrêa et al., 1996), NPC 18521, either given systemically or co-injected topically in association with formalin, attenuated partially but significantly the neurogenic (early) phase and the inflammatory (late) phase of the formalin-induced licking and the oedema formation associated with the late phase of the formalin response. Such results further suggest the participation of bradykinin B_2 receptors

in formalin-induced pain and oedema formation (Corrêa and Calixto, 1993; Corrêa et al., 1996). When compared with the second generation of bradykinin B_2 receptor antagonists such as Hoe 140, NPC 17731 and NPC 17761, and also with the pseudopeptide bradykinin B_2 receptor antagonist NPC 18688, NPC 18521 was more potent and equally efficacious to inhibit both phases of the pain response and the oedema formation after subplantar injection of formalin. As shown for NPC 18688, (Corrêa et al., 1996), NPC 18521 also produced significant inhibition of the neurogenic nociception caused by intraplantar injection of capsaicin into the mouse hindpaw. The small residual agonistic activity observed with higher doses of NPC 18521, in both pain models, was completely prevented by previous treatment with compound 48/80. Such results further confirm the ability of NPC 18521 to induce mast cell degranulation and histamine and/or serotonin release.

Another interesting aspect investigated in the present study was the fact that NPC 18521, although less potent than Hoe 140, when given systemically, produced dose-dependent and complete inhibition of bradykinin-induced hyperalgesia in the rat hindpaw. However, when injected topically into the rat hindpaw, NPC 18521, but not Hoe 140, had a marked hyperalgesic action. This effect was again completely prevented by cyproheptadine, but was not affected by Hoe 140, confirming the mediation of histamine and/or serotonin.

In conclusion, the newly developed pseudopeptide bradykinin B_2 receptor antagonist, NPC 18521, showed potent and long-lasting topical and systemical antioedematogenic and anti-hyperalgesic properties in rats and mice, through an interaction with bradykinin B_2 receptors. Furthermore, in contrast to reports for other bradykinin B_2 recetor antagonists such as Hoe 140, NPC 17731, NPC 17761 and NPC 18688 (Corrêa and Calixto, 1993; Corrêa et al., 1996), the novel pseudopeptide B_2 bradykinin receptor antagonist, NPC 18521, possesses pronounced residual agonistic activity, an action which seems to be secondary to its ability to release histamine and/or serotonin from mast cells.

Acknowledgements

This work was supported by grants from CNPq and FINEP (Brazil). R.O.P. de C. and R.V.A. are undergraduate medical students receiving a grant from CNPq (Proc. 530.471-93-0).

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